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BROMINATED POLYACETYLENIC ACIDS FROM THE MARINE SPONGE *XESTOSPONGIA MUTA*: INHIBITORS OF HIV PROTEASE

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ABSTRACT.—The EtOAc extract of the sponge *Xestospongia muta* collected in Colombus Island, Bahamas, yielded eleven straight-chain unsaturated, polyacetylenic, brominated acids, seven of which were identified on the basis of spectral data, including the unknown acids **2–7**. These acetylenic acids are the first known examples that have been shown to inhibit HIV protease, a critical enzyme in the replication of human immunodeficiency virus.

It is now commonly accepted that human immunodeficiency virus (HIV) is the etiologic agent of acquired immunodeficiency syndrome (AIDS). The clinical successes of azidothymidine (AZT) and dideoxyinosine (DDI) support the approach of controlling this disease by inhibiting the replication of HIV. However, neither drug has proven to be curative, and resistance to AZT as been reported (1). Inhibition of critical viral functions other than reverse transcriptase could have significant utility either in combination therapy or alone.

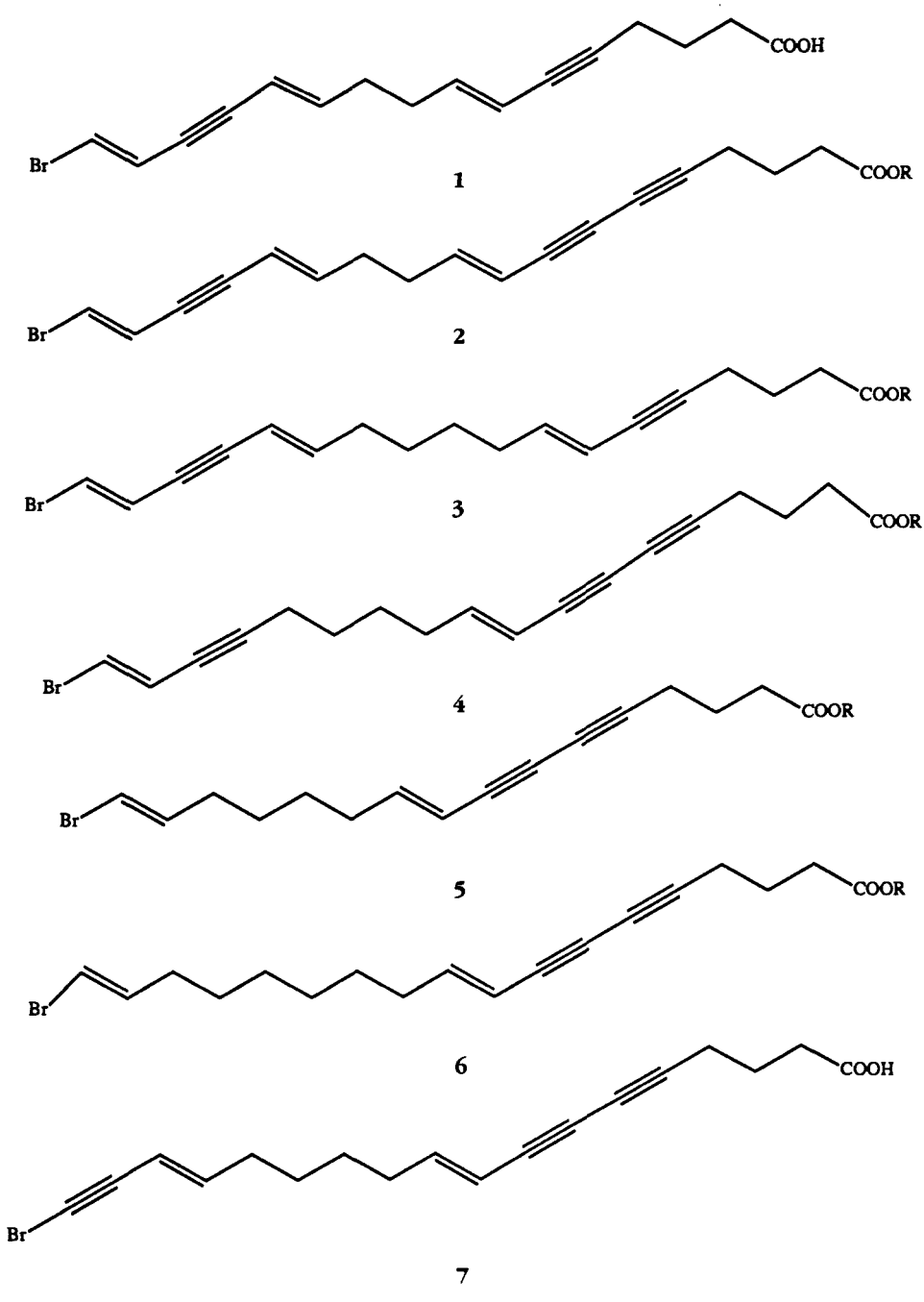
Replication of HIV involves expression of several viral polyproteins that require the presence of a virus-specific protease for their maturation. Inhibition of this enzyme results in immature viral particles and cure of infection in vitro (2). HIV-1 protease is an ideal target for mechanism-based natural product screening in order to identify candidates for development of chemotherapeutics for AIDS (3,4).

There have been a number of reports of the isolation of acetylenic compounds from marine organisms. Only a few of these reports included polyunsaturated, polyacetylenic, or brominated acids (5–7); biological activities of these compounds are primarily antimicrobial (7) and cytotoxic (5).

RESULTS AND DISCUSSION

As part of our continuing search for biologically active metabolites from natural products (8), we encountered the marine sponge *Xestospongia muta* Schmidt (Nepheliospongiidae) collected in Columbus Island. The EtOAc extract of this sponge exhibited significant inhibitory activity in HIV protease assays and as a result was selected for fractionation.

A freeze-dried sample of the sponge *X. muta* was extracted thoroughly with EtOAc to give a brown solid. Si gel cc of this EtOAc extract afforded several fractions showing activity in HIV protease assays. These fractions, after preparative tlc and reversed-phase hplc, provided eleven straight chain, unsaturated, polyacetylenic, brominated acids, seven of which were identified on the basis of spectral data. These are: 16-bromo-(7*E*,11*E*,15*E*)-hexadeca-7,11,15-triene-5,13-diynoic acid [**1**], 18-bromo-(9*E*,13*E*,17*E*)-octadeca-9,13,17-triene-5,7,15-triynoic acid [**2a**], 18-bromo-(7*E*,13*E*,17*E*)-octadeca-7,13,17-triene-5,15-diynoic acid [**3a**], 18-bromo-(9*E*,17*E*)-octadeca-9,17-diene-5,7,15-triynoic acid [**4a**], 16-bromo-(9*E*,15*E*)-hexadeca-9,15-diene-5,7-diynoic acid [**5a**], 18-bromo-(9*E*,17*E*)-octadeca-9,17-diene-5,7-diynoic acid [**6a**], its methyl ester, and 18-bromo-(9*E*,15*E*)-octadeca-9,15-diene-5,7,17-triynoic acid [**7**]. 16-Bromo-(7*E*,11*E*,15*E*)-hexadeca-7,11,15-triene-5,13-diynoic acid [**1**] was reported earlier from the marine sponge *Xestospongia* sp. collected from the Red Sea and was characterized by comparison of spectral data with literature data (7). A second compound, **4a**, was re-



2a–6a R=H
2b–6b R=Me

ported (9) during the preparation of this manuscript. The remaining compounds are reported here for the first time.

Compound **3a**, a colorless, crystalline powder, gave a molecular ion at *m/z* 366/368 $[M + NH_4]^+$ in its cims. The ir spectrum indicated a carboxylic acid (1710 cm^{-1}) and a disubstituted acetylenic group (2210 cm^{-1}). The uv spectrum showed typical fine

structure absorption (228, 272, 283 nm) of a conjugated diene or enyne group (10). The ^{13}C-nmr spectrum (Table 1) contained signals indicative of a bisacetylene group (δ 80.1, 85.5, 89.5, and 91.5 ppm), six methine olefinic carbons (δ 111.0–145.6), and a carbonyl (δ 177.9), which accounted for all of the unsaturation in **1**. The ^{1}H-nmr (pyridine-d_5, Table 2) spectrum of **3a** displayed six olefinic protons at 5.60–6.95 (3 *E* substituted double bonds) while the allylic and other methylene groups occur at δ 1.5–2.5 ppm. Characterization of **3a** as 18-bromo-(7*E*,13*E*,17*E*)-octadeca-7,13,17-triene-5,15-diynoic acid was based mostly on the extensive decoupling experiments and ^{1}H-^{1}H 2D cosy spectra. Irradiation of the olefinic methine proton at δ 6.52 (d, J = 14 Hz, 2.2 Hz) converted the δ 6.95 doublet (J = 14 Hz) to a sharp singlet and removed some of the fine splitting from the δ 5.70 signal, while the irradiation of the methine proton at δ 6.17 (J = 15.7 Hz) caused the δ 5.70 doublet (15.7 Hz) to collapse to a sharp singlet. Similarly, irradiation of the 4H alyllic multiplet at δ 2.12 assigned to the 9- and 12-CH_2 groups caused the 10- and 11-CH_2 multiplet at δ 1.52 to collapse to a broad singlet, while the H-8 and H-13 olefinic multiplets at δ 6.15 and 6.17 turned into sharp doublets because of the absence of allylic coupling, establishing the partial structure of **3a** to be -CH=CH-C≡C-CH=CH-$(CH_2)_4$-CH=CH-X. A methylene triplet next to the acetylenic group at δ 2.31 (J = 7.2 Hz) was coupled only to a methylene at δ 1.72 (quintet, J = 7.4 Hz) which was further coupled to a methylene (triplet, J = 7.4 Hz) at δ 2.47, and chemical shift considerations established that this unit is terminated by a COOH while the bromine atom is at the other end of acid **3a**. Treatment of **3a** with CH_2N_2 gave the methyl ester **3b**, the ir spectrum (1740 cm^{-1}) of which indicated a saturated ester. The ^{1}H-nmr spectrum contained a methyl ester singlet (3H) at δ 3.66 ppm.

Acid **2a**, isolated as an amorphous powder and in a relatively minor amount, had uv and ir spectra very similar to **3a**, suggesting acetylenic and carboxylic acid groups. The molecular ion for **2a** at *m/z* 345/347 $[M+H]^+$ had the elemental composition

TABLE 1. ^{13}C Chemical Shifts For Compounds **2a–6a** and **7** as Determined by DEPT Analysis.

Carbon	Compound					
	2a (MeOH-d_4)	**3a** (Pyridine-d_5)	**4a** (MeOH-d_4)	**5a** (MeOH-d_4)	**6a** ($CDCl_3$)	**7** (MeOH-d_4)
C-1	179.4 s	177.9 s	181.7 s	176.8 s	179.1 s	177.5 s
C-2	36.0 t	33.3 t	37.1 t	33.7 t	33.2 t	34.5 t
C-3	25.7 t	23.6 t	26.6 t	24.2 t	23.0 t	25.1 t
C-4	19.7 t	17.9 t	18.8 t	18.6 t	18.7 t	19.8 t
C-5	83.7[a] s	89.4[a] s	78.3[a] s	82.2[a] s	81.6[a]	79.8[a] s
C-6	74.4[a] s	80.0[a] s	74.4[a] s	73.7[a] s	74.3[a] s	78.4[a] s
C-7	74.0[a] s	110.1[d]	73.8[a] s	72.6[a] s	72.5[a] s	72.8[a] s
C-8	66.5[a] s	143.6 d	66.2[a] s	65.7[a] s	66.4[a] s	66.4[a] s
C-9	111.2 d	31.1[b] t	110.1 d	108.7 d	108.5 d	109.5 d
C-10	147.4 d	30.4[b] t	148.5 d	148.2 d	148.2 d	148.2 d
C-11	33.1 t	29.6[b] t	33.5[b] t	33.0[b] t	32.5[b] t	31.1[b] t
C-12	33.1 t	29.5[b] t	28.8[b] t	28.7[b] t	28.6[b] t	28.9[b] t
C-13	145.3 d	142.2 d	28.9[b] t	28.6[b] t	28.5[b] t	28.8[b] t
C-14	110.0 d	108.8 d	19.1 t	28.5[b] t	28.3[b] t	30.9[b] t
C-15	91.1[a] s	91.2[a] s	93.4[a] s	104.1 d	28.2[b] t	119.1 d
C-16	85.6[a] s	85.5[a] s	83.9[a] s	138.4 d	32.7[b] t	117.9 d
C-17	118.7 d	116.7 d	118.0 d	—	104.0 d	93.5[a] s
C-18	118.9 d	117.4 d	119.1 d	—	137.9 d	84.7[a] s

[a–b]Values in same column with same superscript may be interchanged.

TABLE 2. ^{1}H-nmr Data For Compounds **2a–6a** and **7**.

Proton	Compound					
	2a (MeOH-d_4)	**3a** (Pyridine-d_5)	**4a** (MeOH-d_4)	**5a** (MeOH-d_4)	**6a** ($CDCl_3$)	**7** (Pyridine-d_5)
H-2	2.34 (t, 2H, J = 7.0 Hz)	2.48 (t, 2H, J = 7.4 Hz)	2.33 (t, 2H, J = 7.0 Hz)	2.36 (t, 2H, J = 6.9 Hz)	2.51 (t, 2H, J = 6.7 Hz)	2.52 (t, 2H, J = 6.9 Hz)
H-3	1.80 (quint, 2H, J = 7.2 Hz)	1.72 (quint, 2H, J = 7.0 Hz)	1.78 (quint, 2H, J = 7.3 Hz)	1.79 (quint, 2H, J = 7.0 Hz)	1.86 (quint, 2H, J = 6.8 Hz)	1.98 (quint, 2H, J = 7.1 Hz)
H-4	2.30 (t, 2H, J = 7.0 Hz)	2.31 (t, 2H, J = 7.2 Hz)	2.26 (t, 2H, J = 7.4 Hz)	2.35 (t, 2H, J = 6.9 Hz)	2.41 (t, 2H, J = 6.5 Hz)	2.49 (t, 2H, J = 7.06 Hz)
H-7	—	5.60 (d, 1H, J = 15.7 Hz)	—	—	—	—
H-8	—	6.15 (dt, 1H, J = 15.7, 7.0 Hz)	—	—	—	—
H-9	5.55 (d, 1H, J = 15.9 Hz)	2.12 (m)	5.52 (d, 1H, J = 15.8 Hz)	5.50 (d, 1H, J = 15.5 Hz)	5.46 (d, 1H, J = 15.9 Hz)	5.62 (d, 1H, J = 10.8 Hz)
H-10	6.11 (dt, 1H, J = 15.9, 6.9 Hz)	1.52 (m)	6.20 (dt, 1H, J = 15.8, 6.9 Hz)	6.19 (dt, 1H, J = 15.5, 7.0 Hz)	6.25 (dt, 1H, J = 15.9, 7.4 Hz)	5.99 (dt, 1H, J = 10.8, 7.6 Hz)
H-11	2.21 (m)	1.52 (m)	2.12 (m, 2H)	2.12 (m, 2H)	2.09 (dq, 2H, J = 1.5, 7.1 Hz)	2.19 (m, 2H)
H-12	2.21 (m)	2.12 (m)	1.51 (m)	1.40 (m)	1.37 (m)	1.40 (m)
H-13	6.19 (dt, 1H, J = 15.8, 7.1 Hz)	6.17 (dt, J = 15.7, 7.0 Hz)	1.51 (m)	1.40 (m)	1.29 (m)	1.40 (m)
H-14	5.63 (dd, 1H, J = 15.8, 2.2 Hz)	5.70 (dd, J = 15.7, 2.2 Hz)	2.25 (dt, 2H, J = 7.1, 2.1 Hz)	2.08 (m, 2H)	1.29 (m)	2.29 (m, 2H)
H-15	—	—	—	6.09 (dt, 1H, J = 14.5, 7.1 Hz)	1.37 (m)	6.44 (dt, 2H, J = 14.0, 6.9 Hz)
H-16	—	—	—	6.01 (d, 1H, J = 14.5 Hz)	2.01 (dq, 2H, J = 1.7, 7.2 Hz)	6.94 (d, 1H, J = 14.0 Hz)
H-17	6.35 (dd, 1H, J = 14.0, 2.2 Hz)	6.52 (dd, 1H, J = 14.0, 2.2 Hz)	6.21 (dd, 1H, J = 14.0, 2.1 Hz)	—	6.14 (dt, 1H, J = 13.5, 7.2 Hz)	—
H-18	6.78 (d, 1H, J = 14.0 Hz)	6.95 (d, 1H, J = 14.0 Hz)	6.70 (d, 1H, J = 14.0 Hz)	—	6.02 (d, 1H, J = 13.5 Hz)	—

$C_{18}H_{17}BrO_2$, two hydrogens fewer than **3a**. The ^{1}H nmr (MeOH-d_4) of **2a** exhibited signals for two methylenes α and β to the carboxyl group at δ 2.34 (t, J = 7 Hz) and 1.80 (quintet), respectively, and one methylene group at δ 2.30 (t, J = 7 Hz) adjacent to an acetylenic moiety. The olefinic region in the spectrum displayed a sharp doublet at δ 6.78 (J = 14 Hz) coupled to peaks at δ 6.35 (J = 14.0, 2.2 Hz). Broad doubled triplets at δ 6.11 (J = 15.9, 6.9 Hz) and 6.19 (J = 15.8, 7.0 Hz), each of which was converted to a sharp doublet by irradiation of 4H allylic multiplet (δ 2.21), provided evidence for the same unit as in **3a** with an *E* double bond configuration. However, the ^{13}C-nmr spectrum displayed six carbon signals in the acetylenic carbon region (all s) indicating the presence of three acetylene groups, which also accounted for the additional unsaturation indicated by ms. Presence of only one methylene triplet α to an acetylene group together with the remaining ^{1}H-nmr data suggested that two of the acetylene groups are conjugated. Esterification of **2a** gave a methyl ester **2b**, whose ^{1}H-nmr spectrum had a singlet at δ 3.67 due to the methyl ester group.

Novel triacetylenic acid **4a** was shown to have the indicated structure on the basis of the following evidence. It displayed the molecular ion at *m/z* 347/349 in its cims. The ir, uv, ^{1}H-nmr, and ^{13}C-nmr spectra were very similar to those of the acid **2a** with a few notable exceptions, and in particular, one less unsaturation than **4a**. The ^{1}H-nmr spectrum of **4a** closely paralleled that of **2a**, with assignments based again on the homonuclear 2D nmr spectrum and decoupling experiments. The major difference was replacement of the vinylic protons at δ 6.17 and 5.60 assigned to H-14 and H-13 in **2a** with a doublet of triplets (J = 7, 2.1 Hz) at δ 2.25 and 1.51 assigned to CH_2-14 and CH_2-13 in **4a**. The ^{13}C nmr of **4a** displayed all 18 carbons and closely resembled the spectrum of **2a**, except for the replacement by one methylene carbon at δ 19.1 and 28.9 for two olefinic carbon signals at δ 145.3 and 110.0. DEPT analysis of the ^{13}C-nmr spectrum of **4a** showed 7 quaternary, 4 methine, and 7 methylene carbons. Treatment of **4a** with CH_2N_2 provided its methyl ester **4b**, whose ^{1}H nmr had a methoxyl singlet at δ 3.68.

Diacetylenic acid **6a**, the major acetylenic metabolite isolated from the *X. muta*, which showed modest activity in HIV-protease assays, crystallized readily from MeOH as colorless needles and gave peaks at *m/z* 351/353 $[M+H]^+$ in its cims. The presence of ir bands at 3560–2600 and 1711 and a weak band at 2160 cm^{-1} suggested the presence of carboxylic acid and acetylenic groups, respectively. The uv spectrum showed absorptions at 239, 253, 266, and 283 nm, again characteristic of an endiyne chromophore (11,12). The ^{13}C-nmr spectrum ($CDCl_3$) of **6a** exhibited four signals corresponding to quaternary carbons in two conjugated triple bonds at δ 81.6, 74.3, 72.5, and 66.1 ppm (s) and a carbonyl as a part of the carboxyl group at δ 179.1 (s). The ^{1}H-nmr spectrum of **6a** was slightly different from those of **2a**, **3a**, and **4a**, as seen from the splitting pattern of the olefinic protons. Two doubled triplets at δ 6.25 (J = 15.9, 7.4 Hz) and 6.14 (J = 13.5, 7.2 Hz) assigned to H-10 and H-17 were coupled to two doublets at δ 5.46 (J = 15.8 Hz) and 6.02 (J = 13.5 Hz). A pair of triplets assigned to 4-CH_2 and 2-CH_2, respectively, at δ 2.41 and 2.51 (J = 6.5 and 6.6 Hz) became singlets after irradiation of the 3-CH_2 quintet at δ 1.86. Similarly, the ^{1}H spectrum showed two allylic multiplets (2H each) at δ 2.09 and 2.01, both of which after irradiation resulted in the collapse of multiplets at δ 6.25 and 6.14 into sharp doublets, suggesting that the C-17–C-18 double bond was isolated and not conjugated with an acetylene triple bond. Esterification of **6a** with CH_2N_2 yielded **6b**, as identified from its ir and ^{1}H-nmr spectra and comparison with **6b** isolated in large quantity from the EtOAc extract. Methyl ester **6b** showed strong activity in HIV protease assays (Table 3).

The most abundant acetylenic acid **5a** ($C_{16}H_{19}BrO_2$) was shown to be a lower

TABLE 3. Inhibition of HIV-1 Protease-Catalyzed Proteolysis of Lactate Dehydrogenase by Acetylenic Acids.

Compound	IC_{50} (μM)
2a	6
3a	10
4a	8
6a	12
7	7
6b	10

homologue of **6a** with very similar ir, uv, ^{13}C-nmr, and ^{1}H-nmr spectra except for the broad absorption at δ 1.40 which integrated for four fewer hydrogens, reflecting a shorter alkyl chain. In addition, ^{13}C-nmr signals for sixteen carbons of **5a** appeared at positions identical to those in the spectrum of **6a**, except for the missing methylenes in the aliphatic chain. Acid **5a** gave methyl ester **5b** on treatment with CH_2N_2 and showed a singlet at δ 3.68 in its ^{1}H-nmr spectrum.

The most active compound in HIV protease assays, bromo acetylenic acid **7**, was obtained as a colorless gum and was highly unstable, decomposing rapidly to give a polymeric product that did not move on tlc (Si gel or RP-18). The molecular ion at *m/z* 364/366 $[M+NH_4]^+$ in cims corresponded to the molecular formula $C_{18}H_{19}BrO_2$ (hreims) isomeric with **4a**. The ir spectrum of **7** showed bands consistent with carboxylic acid and acetylenic groups, and the uv spectrum had absorption maxima at 208, 213, 240, 252, and 266 nm characteristic of conjugated enyne functionality. However, the ^{1}H-nmr (pyridine) spectrum was different from that of **4a**. It displayed a CH_2 at δ 2.49 adjacent to an acetylene unit, which was coupled to a 3-CH_2 quintet ($J = 7.1$ Hz) at δ 1.98, and a 2-CH_2 triplet ($J = 6.9$ Hz) at δ 2.52 was different from that of **4a**. Other methylenes appearing in the ^{1}H-nmr spectrum were two allylic CH_2 groups at δ 2.19 and 2.29 ascribed to 11- and 14-CH_2 (as confirmed by decoupling experiments) and a 4H multiplet centered at δ 1.40 which represented the 12- and 13-CH_2 groups of **7**. Lack of an additional methylene group in the region δ 2.4–2.6 conclusively suggested that two of the three acetylene bonds were conjugated with a double bond on one side and the methylene group on the other. The remaining acetylene group had an unsaturation on one side (at C-15–C-16) and a bromine directly attached to acetylene triple bond at the end of the chain as in Br-C≡C-CH=CH-CH_2-.

The ^{13}C-nmr spectrum of **7** is also consistent with the assigned structure, and its DEPT analysis confirmed the number and type of protonated and quaternary carbons. The methyl ester of **7** could not be prepared due to its instability. For these reasons, the proposed structure for compound **7** must be regarded as tentative.

We found these compounds inhibited HIV-1 protease with an IC_{50} of 6 μM to 12 μM (Table 3). The minimal differences in the inhibitory potency within this series of compounds suggests a nonspecific mode of action. To test for general, non-specific inhibition of enzymes, these compounds were tested for inhibition of rat ATP citrate lyase (13) and porcine malic dehydrogenase (14). No inhibition of either enzyme was observed when these compounds were tested at 100 μg/ml. However, extensive cytotoxicity was observed when MOLT-4 cells were exposed to these compounds at a concentration near their IC_{50} values. This lack of specificity for the viral enzyme precludes any further work with these compounds for possible antiviral therapy.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded on a Nicolet Model 20 DXB FTIR spectrometer. ^{1}H- and ^{13}C-nmr spectra, homonuclear correlated 2D spectra, and decoupling experiments were recorded on a Bruker AM-400 spectrometer. Cims and hreims were performed on a Finnigan 4610 and VG 70-VSE instruments. Analytical and preparative tlc were carried out on precoated Si gel G (Kiesel gel G254) and reversed-phase (Whatman KC18F) plates. A Beckman 114M solvent delivery system equipped with a refractive index detector, Model 156, was used for hplc on a Whatman Partisil 10, ODS-3 (Magnum-9) column. Uv spectra were recorded on a Beckman DV-7 spectrophotometer. Reagent grade chemicals (Fisher and Baker) were used.

HIV-1 PROTEASE ASSAY USING LACTATE DEHYDROGENASE AS SUBSTRATE.—The initial velocities of the HIV-1 protease-catalyzed proteolysis of rabbit muscle lactate dehydrogenase (LDH) (obtained from Sigma Chemical Co. and used without further purification) were determined by measurement of the remaining enzymatic activity of LDH following its treatment with HIV-1 protease in the presence of natural product extracts. Reaction mixtures (0.1 ml) containing a solution of the natural product extract, 80 mM sodium acetate (pH 4.7), 1 mM EDTA, 1 mM dithiothreitol, 0.2 M NaCl, and 0.5–2 mg/ml rabbit muscle LDH were preincubated at 37° for 10–60 min with 0.8 μg purified HIV-1 protease. At various times, 5-μl aliquots were withdrawn, diluted into 1 or 2 ml of 0.1 M potassium phosphate (pH 7.0), and incubated at room temperature for 30 min. Remaining LDH activity was then assayed by addition of 100 μl aliquots to 0.9 ml assay mixtures containing 0.1 M potassium phosphate (pH 7.0), 7.6 mM sodium pyruvate, and 0.2 mM NADH (equilibrated at 37°). LDH activity was determined spectrophotometrically by monitoring the continous decrease in NADH absorbance ($\epsilon^{340\ nm} = 6220\ M^{-1}\ cm^{-1}$) using a Perkin-Elmer Lambda 4C spectrophotometer equipped with temperature-regulated cuvette holders equilibrated at 37° or a UVMax spectrophotometer (Molecular Devices). LDH activity was measured by extrapolation of the spectrophotometric rate to zero time to obtain the initial slope. The rate of LDH proteolysis was determined from the percentage of remaining LDH activity and its initial concentration. Control samples contained no protease.

COLLECTION, EXTRACTION, AND ISOLATION.—A sample of the sponge *X. muta* (114 g, dry wt) collected by hand (SCUBA) in Columbus Island, Bahamas, in 1988, was freezed-dried and extracted with EtOAc and MeOH to give 2.6 and 18.5 g of extracts, respectively. Part of the EtOAc extract (0.865 g) that showed strong activity in HIV protease assays was chromatographed over a column of Si gel first using Me_2CO–*n*-hexane (20:80) and finally MeOH-CH_2Cl_2 (5:95). A total of 110 fractions (6–7 ml) were collected and pooled according to their tlc behavior to give 3 bioactive fractions. These fractions after exhaustive repeated preparative tlc and reversed-phase hplc [Whatman column, H_2O-MeOH-TFA (35:65:0.1)] employing a refractive index detector, provided seven pure acids **1** (15 mg, 1.73%), **2a** (13 mg, 1.5%), **3a**, (51 mg, 5.8%), **4a** (21 mg, 2.4%), **5a** (87 mg, 10%), **6a** (17 mg, 1.9%), **7** (14 mg, 1.6%), plus a methyl ester **6b** (11 mg, 1.3%).

18-Bromo-(7E,13E,17E)-octadeca-7,13,17-triene-5,15-diynoic acid [**3a**].—Obtained as colorless, crystalline powder: ir (KBr) 3600–3100, 3000–2800, 2210 (w), 1710, 958 cm^{-1}; λ max (MeOH) 228, 272, 278 nm; ^{1}H nmr see Table 2; ^{13}C nmr see Table 1; cims *m/z* $[M + NH_4]^+$ 366/368, $[M + NH_4 - Br]^+$ 288, $[M + H - HBr]^+$ 269, 179, 119.

18-Bromo-(7E,13E,17E)-octadeca-7,13,17-triene-5,15-diynoic acid methyl ester [**3b**].—An oil: ir (film) 2800, 2208, 1728, 960 cm^{-1}; λ max (hexane) 228, 273, 288 nm; ^{1}H nmr ($CDCl_3$) δ 6.96 (d, 1H, $J = 14.2$ Hz), 6.54 (dd, 1H, $J = 14.2, 2.2$ Hz), 6.18 (dt, 1H, $J = 15.6, 7.0$ Hz), 6.16 (dt, 1H, $J = 15.8, 7.0$ Hz), 5.71 (dd, 1H, $J = 15.6, 2.2$ Hz), 5.60 (d, 1H, $J = 15.8$ Hz), 3.66 (s, 3H), 2.46 (t, 2H, $J = 7.3$ Hz), 2.32 (t, 2H, $J = 7.12$ Hz), 2.13 (m), 1.73 (quint, 2H, $J = 7.1$ Hz) 1.51 ppm (m); hreims *m/z* 362.

18-Bromo-(9E,13E,17E)-octadeca-9,13,17-triene-5,7,15-triynoic acid [**2a**].—Amorphous powder: ir (KBr) 3600–3100, 3000–2850, 2215 (w), 1715 cm^{-1}; λ max (MeOH) 227, 248, 275, 288 nm; ^{1}H nmr see Table 2; ^{13}C nmr see Table 1; cims *m/z* $[H + NH_4]^+$ 362/364. *Anal.* calcd for $C_{18}H_{17}BrO_2$ *m/z* 344.024123; found 344.025181.

18-Bromo-(9E,13E,17E)-octadeca-9,13,17-triene-5,7,15-triynoic acid methyl ester [**2b**].—Colorless gum: ir (CCl_4) 2845, 2216, 1730 cm^{-1}; λ max (hexane) 227, 248, 275, 288; ^{1}H nmr ($CDCl_3$) δ 6.75 (d, 1H, $J = 14.0$ Hz), 6.33 (dd, 1H, $J = 14.0, 2.2$ Hz), 6.19 (dt, 1H, $J = 15.7, 7.0$ Hz), 6.11 (dt, 1H, $J = 15.9, 6.8$ Hz), 6.38 (dd, 1H, $J = 14.0, 2.2$ Hz), 5.62 (dd, 1H, $J = 15.7, 2.2$ Hz), 5.53 (d, 1H, $J = 15.9$ Hz), 3.68 (s, 3H), 2.33 (t, 2H, $J = 7.0$ Hz), 2.31 (t, 2H, $J = 7.0$ Hz), 2.21 (m), 1.79 ppm (quint, 2H, $J = 7.2$ Hz); hreims *m/z* 358.

18-Bromo-(9E,17E)-octadeca-9,17-diene-5,7,15-triynoic acid [**4a**].—White crystalline powder: ir

(KBr) 3600–3100, 3100–3000, 2800, 2220 (W), 1712, 1626, 1560, 1439, 925 cm^{-1}; λ max (MeOH) 234, 252, 225, 285 nm; ^1H nmr see Table 2; ^{13}C nmr see Table 1; cims *m/z* $[M+H]^+$ 347/349.

18-Bromo-(9E,17E)-octadeca-9,17-diene-5,7,15-triynoic acid methyl ester [**4b**].—An oil: ir (CCl_4) 2810, 2210, 1730, 1630, 1560, 1440, 915 cm^{-1}; λ max (hexane) 233, 249, 275, 288 nm; ^1H nmr ($CDCl_3$) δ 6.69 (d, 1H, J = 14.2 Hz), 6.20 (dd, 1H, J = 14.2, 2.1 Hz), 6.19 (dt, 1H, J = 15.8, 6.9 Hz), 5.51 (d, 1H, J = 15.8 Hz), 3.68 (s, 3H), 2.34 (t, 2H, J = 7.0 Hz), 2.25 (t, 2H, J = 7.4 Hz), 2.24 (dt, 2H, J = 7.1, 2.1 Hz), 2.12 (m, 2H), 1.79 (quint, 2H, J = 7.2 Hz), 1.50 ppm (m, 4H); lreims *m/z* 360.

18-Bromo-(9E,17E)-octadeca-9,17-diene-5,7-diynoic acid [**6a**].—Colorless needles, ir (neat) 3600–3100, 3100–3000, 3000–2800, 2218 (w), 1710, 1635, 1619, 1202 cm^{-1}; λ max (MeOH) 239, 253, 266, 283 nm; ^1H nmr see Table 2; ^{13}C nmr see Table 1; cims *m/z* $[M+H]^+$ 351/353.

18-Bromo-(9E,17E)-octadeca-9,17-diene-5,7-diynoic acid methyl ester [**6b**].—Yellow gum: ir (CCl_4) 2800, 2218, 1730, 1635, 1620, 1205 cm^{-1}; λ max (hexane) 240, 253, 267, 282 nm; ^1H nmr ($CDCl_3$) δ 6.25 (dt, 1H, J = 15, 9, 7.4 Hz), 6.13 (dt, 1H, J = 13.8, 7.1 Hz), 6.00 (d, 1H, J = 13.8 Hz), 5.46 (d, 1H, J = 15.9 Hz), 3.67 (s, 3H), 2.50 (t, 2H, J = 6.8 Hz), 2.40 (t, 2H, J = 6.7 Hz), 2.10 (m, 4H), 1.85 (quint, 2H, J = 6.8 Hz), 1.37 (m, 4H), 1.25 ppm (m, 4H); lreims *m/z* 364.

16-Bromo-(9E,15E)-hexadeca-9,15-diene-5,7-diynoic acid [**5a**].—Amorphous powder: ir (KBr) 3600–3100, 3100–3000, 3000–2800, 2215 (w) 1709, 1630, 1620, 1200 cm^{-1}; λ max (MeOH) 234, 247, 267, 282 nm; ^1H nmr see Table 2; ^{13}C nmr see Table 1; cims *m/z* $[M+H]^+$ 323/325.

16-Bromo-(9E,15E)-hexadeca-9,15-diene-5,7-diynoic acid methyl ester [**5b**].—Colorless gum: ir (CCl_4) 2800, 2213, 1728, 1630, 1621, 1205 cm^{-1}; λ max (hexane) 235, 250, 265, 283 nm; ^1H nmr ($CDCl_3$) δ 6.18 (dt, 2H, J = 15.5, 7.01), 6.08 (dt, 1H, J = 14.6, 7.1 Hz), 6.00 (d, 1H, J = 14.6 Hz), 5.52 (d, 1H, J = 15.5 Hz), 3.67 (s, 3H), 2.35 (t, 2H, J = 6.9 Hz), 2.34 (t, 2H, J = 6.9 Hz), 2.12 (m, 2H), 2.08 (m, 2H), 1.78 (quint, 2H, J = 7.0 Hz), 1.39 ppm (m, 4H); lreims *m/z* 336.

18-Bromo-(9E,15E)-octadeca-9,15-diene-5,7,17-triynoic acid [**7**].—An unstable brown powder which decomposed on storage: ir (KBr) 3600–3100, 3000–2800, 2221 (w), 1710 cm^{-1}; λ max (MeOH) 208, 213, 240, 252, 266 nm; ^1H nmr see Table 2; ^{13}C nmr see Table 1; cims $[M+NH_4]^+$ 364/365, $[M+NH_4-HBr]^+$ 267. *Anal.* calcd for $C_{18}H_{20}BrO_2$ *m/z* $[M+H]^+$ 347.064666; found 347.062848.

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